CLAIMS

1.- A method of efficient production of high levels of the recombinant sucrose synthase (SS) enzyme in its soluble, active form, characterized in that it comprises:

a) Obtaining, from the nucleotide sequence of the gene that encodes the isoform of SS of potato SS4, 2 primers, represented by SEQ ID NO: 1 and SEQ ID NO: 2, with which, on the basis of a potato leaf cDNA library, a cDNA fragment of 2418 base pairs represented by SEQ ID NO: 3 is amplified by PCR

b) Inserting said cDNA fragment in a first vector

c) Inserting said first vector in a first host microorganism where it is amplified

d) Digesting the amplified construction with at least 2 restriction enzymes

e) Obtaining, after prior digestion, a DNA fragment containing the cDNA that encodes SSX, the deducted amino acid sequence of which is represented by SEQ ID NO: 4

f) Cloning said fragment at the same restriction sites as a vector that contains a nucleotide sequence encoding a histidine-rich sequence, fusing said histidine-rich tail to SS, giving rise to a 2nd expression vector.

g) Inserting this 2nd vector in a 2nd host microorganism where it is expressed

h) Incubating said transformed microorganism in suitable culture conditions for the synthesis of SSX in its soluble, active form

i) Isolating and purifying SSX in its active form

2.- The method of production of recombinant SS as claimed in the preceding claim, characterized in that the first expression vector used in stage b) is the plasmid pSK, which when inserted in SEQ ID NO: 3 gives rise to the construction pSS of Fig. 3A.

3.- The method of production of recombinant SS as claimed in the preceding claim, characterized in that the first host microorganism used in stage c) for amplifying the pSK plasmid is the *E. coli* bacterium XL1 Blue.

4.- The method of production of recombinant SS as claimed in any one of the preceding claims, characterized in that the restriction enzymes used in stage d) are NcoI and NotI.

5.- The method of production of recombinant SS as claimed in any one of the preceding claims, characterized in that, in stage f), the restriction sites where the DNA fragment released after stage d) is cloned, are the same as the plasmid pET-28a(+), shown in Fig. 3B, giving rise to the pET-SS plasmid shown in Fig. 3C.

6.- The method of production of recombinant SS as claimed in any one of the preceding claims, characterized in that the 2nd host microorganism used in stage g) is the BLR(DE3) strain of *Escherichia coli*.

7.- The method of production of recombinant SS as claimed in any one of the preceding claims, characterized in that the strain transformed in stage g) which is incubated in stage h) in suitable culture conditions for the synthesis of SSX in its soluble and active form, is CECT5850.

8.- The method of production of recombinant SS as claimed in any one of the preceding claims, characterized in that the suitable culture conditions for the synthesis of SSX comprise submitting the bacterial culture to a temperature of 20°C.

9.- The method of production of recombinant SS as claimed in any one of the preceding claims,

characterized in that purification of SSX is preferably effected by affinity chromatography for amino acid sequences rich in histidine residues with an elution buffer that contains imidazole, preferably, at a concentration of 200 mM.

10.- The method of production of recombinant SS as claimed in any one of the preceding claims, characterized in that, in order to maintain the purified SSX enzyme in its active form, said purified enzyme eluted from affinity chromatography is immediately submitted to dialysis to remove any trace of imidazole.

11.- A method of production of a recombinant potato SS5 isoform, characterized in that it comprises:

a) Using the construction pSS as template and, by directed mutagenesis after successively using the pairs of primers whose sequences are [SEQ ID NO: 5, SEQ ID NO: 6], [SEQ ID NO: 7, SEQ ID NO: 8] and [SEQ ID NO: 9, SEQ ID NO: 10], obtaining a DNA fragment of 2418 base pairs represented by SEQ ID NO: 11

b) Inserting said DNA fragment in a first vector

c) Inserting said first vector in a first host microorganism where it is amplified

d) Digesting the amplified construction with at least 2 restriction enzymes

e) Obtaining, after prior digestion, a DNA fragment that encodes SS5, the amino acid sequence of which is represented by SEQ ID NO: 12

f) Cloning said fragment at the same restriction sites as a vector that contains a nucleotide sequence encoding a His-rich sequence, fusing said His-rich tail to SS5, giving rise to a 2nd expression vector.

g) Inserting this 2nd vector in a 2nd host microorganism where it is expressed

h) Incubating said transformed microorganism in suitable culture conditions for the synthesis of SS5 in its soluble, active form

i) Isolating and purifying SS5 in its active form

12.- The method of production of recombinant SS5 as claimed in claim 11, characterized in that stage b) gives rise to the construction pSS5.

13.- The method of production of recombinant SS5 as claimed in either one of the claims 11 or 12, characterized in that the first host microorganism used in stage c) for amplifying pSS5 is the *E. coli* bacterium XL1 Blue.

14.- The method of production of recombinant SS5 as claimed in any one of the claims 11 to 13, characterized in that the restriction enzymes used in stage d) are NcoI and NotI.

15.- The method of production of recombinant SS5 as claimed in any one of the claims 11 to 14, characterized in that, in stage f), the restriction sites where the DNA fragment released after stage d) is cloned, are the same as the plasmid pET-28a(+) giving rise to the plasmid pET-SS5.

16.- The method of production of recombinant SS5 as claimed in any one of the claims 11 to 15, characterized in that the 2nd host microorganism used in stage g) is the BLR(DE3) strain of *Escherichia coli*.

17.- The method of production of recombinant SS5 as claimed in any one of the claims 11 to 16, characterized in that the strain transformed in stage g) which is incubated in stage h) in suitable culture conditions for the synthesis of SS5 in its soluble and active form, is CECT5849.

18.- The method of production of recombinant SS5 as claimed in any one of the claims 11 to 17, characterized in that the suitable culture conditions for the synthesis of SS5 comprise submitting the bacterial culture to a temperature of 20°C.

19.- The method of production of recombinant SS5 as claimed in any one of the claims 11 to 18, characterized in that purification of SS5 is preferably effected by affinity chromatography with an elution buffer that contains imidazole, preferably, at a concentration of 200 mM.

20.- The method of production of recombinant SS5 as claimed in any one of the claims 11 to 19, characterized in that, in order to maintain the purified SS5 enzyme in its active form, said purified enzyme eluted from affinity chromatography is immediately submitted to dialysis to remove any trace of imidazole.

21.- A soluble, active recombinant SSX enzyme product, obtainable as claimed in the method of claims 1 to 10, characterized in that it has a deducted sequence represented by SEQ ID NO: 4 and displays sucrose synthase (SS) activity.

22.- The soluble, active recombinant SSX enzyme product as claimed in claim 21, characterized in that it has a specific activity of 80 U/mg protein in the presence of sucrose and UDP, and kinetic parameters of Km(UDP) = 0.25 mM and Km(sucrose) = 30 mM.

23.- A SS5 isoform of the soluble, active recombinant enzyme product of claims 21 and 22, obtainable as claimed in the method of claims 11 to 20, characterized in that it has a deducted amino acid sequence shown in SEQ ID NO: 12 and displays sucrose synthase (SS) activity.

24.- The recombinant SS5 isoform as claimed in claim 23, characterized in that it has a specific activity of 80 U/mg protein and 60 U/mg protein in the presence of UDP and ADP, respectively, and kinetic parameters with respect to UDP and ADP of Km(UDP) = Km(ADP) = 0.3 mM.

25.- The use of the enzyme product of claims 21 and 22 in the production of UDPG, characterized by incubation of UDP and SSX in suitable conditions followed by isolation and purification of the UDPG produced.

26.- Use as claimed in claim 25, characterized in that it comprises:

a) Incubating 100 ml of the following solution for 12 h at 37°C:

Sucrose	1 M
HEPES, pH 7.0	50 mM
EDTA	1 mM
Polyethylene glycol	20%
$MgCl_2$	1 mM
KCl	15 mM
UDP	100 mM
SSX	30 U

b) Stopping the reaction by heating, preferably at 100°C for 90 s

c) Centrifuging at 10000 g for 10 min

d) Chromatographing the supernatant by HPLC, eluting and purifying the UDPG by conventional methods.

27.- The use of the enzyme product of claims 23 and 24 in the production of ADPG, characterized by incubation of ADP and SS5 in suitable conditions, followed by isolation and purification of the ADPG produced.

28.- The use as claimed in claim 27, characterized in that it comprises:

e) Incubating 100 ml of the following solution for 12 h at 37°C:

Sucrose	1 M
HEPES, pH 7.0	50 mM
EDTA	1 mM
Polyethylene glycol	20%
$MgCl_2$	1 mM
KCl	15 mM
ADP	100 mM

f) Stopping the reaction by heating, preferably at 100°C for 90 s

g) Centrifuging at 10000 g for 10 min

h) Chromatographing the supernatant by HPLC, eluting and purifying the ADPG by conventional methods.

29.- The use of an enzyme product with sucrose synthase (SS) activity in the manufacture of assay kits for the spectrophotometric/fluorimetric/amperometric determination of sucrose.

30.- The use as claimed in claim 29, characterized in that it comprises the following incubation medium:

a. 2 units of SS

b. 2 mM of ADP

c. 2 units of ADPG pyrophosphatase of plant, animal or microbial origin

d. 2 units of PGM

e. 2 units of G6PDH

f. 0.5 mM of NAD(P)

g. 100 ml of reaction buffer: 50 mM HEPES, pH 7.0 / 1 mM EDTA / 20% polyethylene glycol / 1 mM $MgCl_2$ / 15 mM KCl

h. previously filtered test sample.

31.- The use as claimed in claim 29, characterized in that it comprises the following incubation medium:
a. 2 units of SS
b. 2 mM of UDP
c. 2 units of UDPG pyrophosphatase of plant, animal or microbial origin
d. 2 units of PGM
e. 2 units of G6PDH
f. 0.5 mM of NAD(P)
g. 100 ml of reaction buffer: 50 mM HEPES, pH 7.0 / 1 mM EDTA / 20% polyethylene glycol / 1 mM $MgCl_2$ / 15 mM KCl
h. previously filtered test sample.

32.- The use as claimed in claim 29, characterized in that it comprises the following incubation medium:
a. 2 units of SS
b. 2 mM of UDP
c. 2 units of UDPG dehydrogenase
d. 0.5 mM of NAD
e. 100 ml of reaction buffer: 50 mM HEPES, pH 7.0 / 1 mM EDTA / 20% polyethylene glycol / 1 mM $MgCl_2$ / 15 mM KCl
f. previously filtered test sample.

33.- The use as claimed in any one of the claims 29 to 32, characterized in that the SS present in the assay kit is, indiscriminately, SS4, SS5 or SSX or a combination thereof.

34.- The use of DNA that encodes SS, in the production of transgenic plants that express SS, characterized by inserting a genetic construction that contains and expresses said DNA in a suitable vector and transferring said genetic construction to the genome of a plant.

35.- The use as claimed in claim 34, characterized in that the cDNA used encodes SSX.

36.- The use as claimed in claim 35, characterized in that it comprises the following steps:

a) Successive insertion, in the pSS plasmid, of the promoter 35S and of the terminator NOS in the 5' and 3' regions, respectively, of the *SSX* gene or any other version that encodes SS, to produce the plasmid p35S-SS-NOS, the restriction map of which is shown in Fig. 4B

b) Successive digestion of p35S-SS-NOS with the enzymes NotI, T4 DNA polymerase and HindIII

c) Cloning of the fragment produced, within the binary plasmid pBIN20, previously digested successively with EcoRI, T4 DNA polymerase and HindIII, obtaining the plasmid pBIN35S-SS-NOS shown in Fig. 4C

d) Amplification of pBIN35A-SS-NOS in *E. coli* (XL1 Blue)

e) Insertion of the genetic construction amplified in the preceding stage in *Agrobacterium tumefaciens* C58:GV2260, obtaining a transformed strain CECT 5851

f) Transfection of plants with the transformed strain CECT 5851.

37.- The use as claimed in claim 35, characterized in that it comprises the following steps:

a) Successive insertion, in the pGEMT plasmid, of the promoter of the gene that encodes the small subunit of RUBISCO for producing the plasmid pGEMT-RBCSprom, the restriction map of which is shown in Fig. 5A

b) Digestion of pGEMP-RBCS with the enzymes HindIII and NcoI for inserting the fragment released at the corresponding restriction sites of p35S-SS-NOS, giving rise to pRBCS-SS-NOS, the restriction map of which is shown in Fig. 5B.

c) Successive digestion of pRBCS-SS-NOS with HindIII, T4 DNA polymerase and NotI and cloning of the fragment released in pBIN20 digested successively with HindIII, T4 DNA polymerase and EcoRI, giving rise to pBINRBCS-SS-NOS, the restriction map of which is shown in Fig. 5C.

d) Amplification of pBINRBCS-SS-NOS in *E. coli* (XL1 Blue)

e) Insertion of the genetic construction amplified in the preceding stage in *Agrobacterium tumefaciens* C58:GV2260 and transfection of plants with the transformed strain.

38.- Transgenic plants obtainable by the method of use of claims 33 to 37, characterized by overexpression of an SS enzyme activity.

39.- The transgenic plants as claimed in claim 37, characterized in that said overexpression assumes levels of SS enzyme activity of the order of 2-10 times greater than those present in the same tissue of a non-transgenic wild-type plant.

40.- The transgenic plants as claimed in claims 38 or 39, characterized in that they are preferably selected from tobacco, potato, tomato or rice plants.

41.- The transgenic plants as claimed in any one of the claims 38 to 40, characterized in that in addition they have higher contents of sucrose, G6P, ADPG and starch, than those observed in the same tissue or organ of the corresponding wild-type plants, grown in identical conditions.

42.- The transgenic plants as claimed in claim 40, the leaves of which have a content of sucrose, G6P, ADPG and starch, and an amylose/amylopectin ratio, higher than those observed in the leaves of the corresponding wild-type plants.

43.- The transgenic plants as claimed in claim 40, whose roots, tubers and/or seeds have a content of sucrose, G6P, ADPG and starch, and an amylose/amylopectin ratio, higher than those observed in the same tissues or organs of the corresponding wild-type plants.